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(54) **RECEPTEURS DE RETINOIDES X ET COMPOSANTS DE LA MACHINE DE TRANSCRIPTION BASALE**

(54) **RETINOID X RECEPTORS AND COMPONENTS OF THE BASAL TRANSCRIPTION MACHINERY**

(57) Le recepteur de retinoide X (RXR) participe a une large gamme de chemins de signalisation hormonale soit comme homodimere soit comme heterodimere avec d'autres membres de la superfamille des recepteurs que sont les hormones steroide/thyroide. Selon cette invention, la fonction de trans-activation du RXR, qui est dépendante des ligands, a été caractérisee et l'aptitude du RXR à interagir avec des composants de la machine de transcription basale a été examinee. Des expérimentations in vivo et in vitro indiquent que le domaine de fixation des ligands du RXR établit un contact direct spécifique et dépendant des ligands avec une région hautement conservée de la proteine de fixation de TATA (TBP). L'aptitude qu'ont les mutations qui réduisent la transcription dependante des ligands par RXR a rompre l'interaction RXR-TBP in vivo et in vitro laisse supposer que le RXR établit un contact direct avec la machine de transcription basale, afin d'en assurer l'activation.

(57) The retinoid X receptor (RXR) participates in a wide array of hormonal signaling pathways either as a homodimer or as a heterodimer with other members of the steroid/thyroid hormone superfamily of receptors. In accordance with the present invention, the ligand-dependent transactivation function of RXR has been characterized and the ability of RXR to interact with components of the basal transcription machinery has been examined. In vivo and in vitro experiments indicate the RXR ligand binding domain makes a direct, specific and ligand-dependent contact with a highly conserved region of the TATA binding protein (TBP). The ability of mutations that reduce ligand-dependent transcription by RXR to disrupt the RXR-TBP interaction in vivo and in vitro suggests that RXR makes direct contact with the basal transcription machinery in order to achieve activation.

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(54) Title: RETINOID X RECEPTORS AND COMPONENTS OF THE BASAL TRANSCRIPTION MACHINERY

(57) Abstract

The retinoid X receptor (RXR) participates in a wide array of hormonal signaling pathways either as a homodimer or as a heterodimer with other members of the steroid/thyroid hormone superfamily of receptors. In accordance with the present invention, the ligand-dependent transactivation function of RXR has been characterized and the ability of RXR to interact with components of the basal transcription machinery has been examined. *In vivo* and *in vitro* experiments indicate the RXR ligand binding domain makes a direct, specific and ligand-dependent contact with a highly conserved region of the TATA binding protein (TBP). The ability of mutations that reduce ligand-dependent transcription by RXR to disrupt the RXR-TBP interaction *in vivo* and *in vitro* suggests that RXR makes direct contact with the basal transcription machinery in order to achieve activation.

Retinoid X receptors and components of the basal transcription machinery

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FIELD OF THE INVENTION

The present invention relates to methods for the modulation of nuclear receptor mediated processes. In a particular aspect, the present invention relates to methods for the identification of compounds useful for such modulation, as well as compositions useful for such assays.

BACKGROUND OF THE INVENTION

Members of the steroid/thyroid hormone superfamily of receptors regulate expression of complex gene networks involved in vertebrate development, differentiation and homeostasis. A defining characteristic of these receptors lies in part in their ability to function as ligand-activated transcription factors. Retinoid X receptors (RXRs) occupy a central position in the function and activity of many members of this superfamily of receptors. For example, by forming heterodimers with retinoic acid receptors (RARs), thyroid hormone receptors (TRs), vitamin D receptors (VDRs), peroxisome proliferator activated receptors (PPARs) and several orphan receptors, RXRs participate in a diverse array of signaling pathways (Mangelsdorf et al., *Recent Prog. in Hormone Res.* 48:99-121 (1993)). The ability of

RXR homodimers to respond to 9-*cis* retinoic acid identifies still another signaling pathway influenced by this nuclear receptor. The critical role of RXRs in the function of nuclear receptors is further highlighted by the structural and functional conservation between vertebrate RXRs and the *Drosophila* nuclear receptor *ultraspiricle* (Oro et al., *Nature* 347:298-301 (1990); and Yao et al., *Cell* 71:63-72 (1992)).

The mechanism by which RXR (and other nuclear receptors) activates transcription is poorly understood. Numerous studies have defined two independent transactivation functions (tau domains; τ) in most members of the steroid/thyroid hormone superfamily of receptors. These activation functions include a constitutive activation function (τ1 or AF-1) present in the amino-terminal region and a ligand-dependent activation function (τc or AF-2) present in the carboxy-terminal 200-250 amino acids. The carboxy-terminal domain of nuclear receptors is complex, mediating ligand-dependent activation, receptor homo- and heterodimerization and ligand binding (Parker, M.G., *Curr. Opin. in Cell Biol.* 5:499-504 (1993); and Stunnenberg, H.G., *BioEassays* 15:309-315 (1993)). Binding of ligand is thought to induce a conformational change in receptors that leads to activation of transcription (Allan et al., *J. Biol. Chem.* 267:19513-19520 (1992); Beekman et al., *Mol. Endrocrinol.* 7:1266-1274 (1993); Toney et al., *Biochemistry* 32:2-6 (1993); Leid, M., *J. Biol. Chem.* 269:14175-14181 (1994)).

It is not currently known how activated receptors propagate their signals to the basal transcription machinery. Direct interactions between the basal transcription factor TFIIB and several nuclear receptors have been reported (Ing et al., *J. Biol. Chem.* 267:17617-17623 (1992); Baniahmad et al., *Proc. Natl. Acad. Sci. USA* 90:8832-8836 (1993); Fondell et al., *Genes & Devel.* 7:1400-

1410 (1993); Blanco et al., *Proc. Natl. Acad. Sci. USA* **92**:1535-1539 (1995); and MacDonald et al., *J. Biol. Chem.* **270**:4748-4752 (1995)). The nuclear receptor-TFIIB interaction does not appear to be influenced by ligand. Indeed, it has been suggested that interaction between TR and TFIIB may be associated with transcriptional repression (Baniahmad et al., (1993) supra; and Fondell et al., supra). The identification of several novel proteins suggested to be involved in ligand-activated transcription by nuclear receptors (Halachmi et al., *Science* **264**:1455-1458 (1994); Jacq et al., *Cell* **79**:107-118 (1994); Berkanstam et al., *Cell* **69**:401-412 (1992); Cavailles et al., *Proc. Natl. Acad. Sci. USA* **91**:10009-10013 (1994); and Lee et al., *Nature* **374**:91-94 (1995)) suggests that coactivators or bridging factors may also be involved in transmitting the signal from ligand activated receptors to the basal transcription apparatus.

In view of the limited understanding of how activated receptors propagate their signals to the basal transcription machinery, what is needed in the art is a better understanding of such signalling processes. The identification of components of the basal transcription machinery involved in such signalling would be of great value. The identification of such components would also facilitate the development of assays for novel ligands for nuclear receptors.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified components of the basal transcription machinery involved in RXR signalling. Further in accordance with the present invention, the ligand-dependent transactivation function of RXR has been characterized and the ability of RXR to interact with components of the basal transcription machinery has been examined. *In vivo* and *in vitro*

experiments indicate the RXR ligand binding domain makes a direct, specific and ligand-dependent contact with a highly conserved region of the TATA binding protein (TBP). The ability of mutations that reduce ligand-dependent transcription by RXR to disrupt the RXR-TBP interaction *in vivo* and *in vitro* suggests that RXR makes direct contact with the basal transcription machinery in order to achieve activation.

Recently a small region at the carboxy terminus of RXR has been identified that is required for ligand-activated transcription (Durand et al., *EMBO J.* **13**:5370-5382 (1994); Leng et al., *Mol. Cell. Biol.* **15**:255-263 (1995); and Zhang et al., *Mol. Cell. Biol.* **14**:4311-4323 (1994)). This activation domain (τc), which is conserved among most members of the steroid and thyroid hormone receptor superfamily (Danielian et al., *EMBO J.* **11**:1025-1033 (1992)), functions as a constitutive activator when fused to a heterologous DNA binding domain. In accordance with the present invention, the transactivation properties of RXR have been examined in both mammalian and in *Saccharomyces cerevisiae* cells. The ability of the RXR τc domain to function in both mammalian cells and in *S. cerevisiae* suggests that activation pathways mediated by RXR are conserved. Both *in vivo* and *in vitro* experiments indicate the RXR τc domain mediates an interaction between the RXR ligand binding domain and the conserved carboxy-terminal domain of the TATA binding protein (TBP). Mutations in either the RXR τc domain or in TBP disrupt this interaction, suggesting that the RXR-TBP interaction plays a functional role in transactivation by RXR.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the effect of point mutations in the RXR tc domain on the ability of RXR to induce transactivation.

Figure 1A presents transactivation results with fusions between the GAL4 DNA binding domain and the last 19 amino acids of human RXRα (amino acids 444-462) and the last 20 amino acids of human TRα (amino acids 391-410). Bold letters identify the mutations introduced into the RXR444-462 sequence. Dotted lines indicate all other amino acids are identical to the RXR444-462 sequence. The activity of GAL4RXR444-462 was set at 100%.

Figure 1B presents transactivation results with constructs prepared by introducing the point mutations described in Figure 1A into the GAL4-RXR ligand binding domain fusion (GAL4RXR197-462). GAL4RXR197-443 represents the tc truncation. After transfection, CV1 cells were cultured in the presence (filled bars) or absence (open bars) of 100 nM LG69 (an RXR specific ligand) for 36 hours. Fold induction relative to the reporter alone is reported.

Figure 2 presents results of the yeast two-hybrid assay to assess the interaction between receptor ligand binding domains and various components of the basal transcription machinery.

Figure 2A presents results obtained employing fusions between the GAL4 activation domain and RXR, RAR, and TR; activation domain fusions were cotransformed into the strain Y190 along with fusions between the GAL4 DNA binding domain and the conserved carboxy terminal domain of human TBP. The results presented herein illustrate the interaction between receptor ligand binding domains and TBP. The activity of the GAL4 activation domain alone was

measured only in the absence of ligand. Beta-galactosidase activity was measured after growth for 16 hours in the presence (filled bars) or absence (open bars) of 1 μM 9-*cis* retinoic acid (RXR and RAR) or 1 μM TRIAC (TR). No interaction between receptor and TBP is detected in the absence of ligand.

Figure 2B illustrates the interaction between RXR ligand binding domain mutants and TBP. Only activity in the presence of 9-*cis* retinoic acid is shown. No interaction between the mutants and TBP is detected in the absence of 9-*cis* retinoic acid. Point mutants consist of amino acids 197-462 of RXR. RXR197-443 represents the rc truncation.

Figure 2C repeats the experiments summarized in Figure 2A, using full length *Drosophila* TAF110 in place of human TBP.

Figure 2D illustrates the interaction between RXR ligand binding domain mutants and TAF110. Only activity in the presence of 9-*cis* retinoic acid is shown. No interaction between the mutants and TAF110 is detected in the absence of 9-*cis* retinoic acid.

Figure 3 demonstrates th point mutations in the basic repeat of TBP disrupt the interaction with RXR *in vivo*.

Figure 3A presents results when a fusion between the GAL4 activation domain and RXR (amino acids 197-462) was cotransformed into a host (strain Y190) along with fusions between the GAL4 DNA binding domain and human TBP (the conserved carboxy terminal domain, amino acids 151-335). Y233G, R321E/K232E/R235E, V236G and V237G identify the amino acid changes introduced into TBP. Beta-galactosidase activity was measured after growth for 16

hours in the presence of 1 μM 9-*cis* retinoic acid as described in the Example section.

Figure 3B presents results when fusions between the GAL4 activation domain and RXR rc mutants were cotransformed into a host (strain Y190) along with a fusion between the GAL4 DNA binding domain and the TBP mutant V237G described in reference to Figure 3A. Beta-galactosidase activity was measured after growth for 16 hours in the presence (filled bars) or absence (open bars) of 1 μM 9-*cis* retinoic acid as described in the Example section. Point mutants consist of amino acids 197-462 of RXR. RXR197-443 represents the rc truncation. Note the difference in scale between A and B.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, mutations of the ligand-dependent activation function (rc) of RXR are exploited to examine the role of this domain in ligand-dependent transactivation. The rc domain encodes a potential amphipathic alpha helix with hydrophobic and negatively charged faces. This domain is necessary for ligand-dependent activation of transcription by RXR and is sufficient to activate transcription when fused to a heterologous DNA binding domain in both mammalian cells and *S. cerevisiae* (see Figure 1). Using both the yeast two-hybrid assay and *in vitro* GST pull-down experiments, the RXR ligand binding domain has been shown to make a direct and specific contact with the basic repeat present in the conserved carboxy-terminal domain of the TATA binding protein (TBP; see Figures 2 and 3). The ability of mutations in the rc domain that reduce the transactivation ability of RXR to disrupt the RXR-TBP interaction *in vivo* and *in vitro* suggests this interaction has functional significance.

Both RXR and TR interact with a second component of the TFIID complex, TAF110 (see Figure 2B). The finding that a functional AD domain is not required for the RXR-TAF110 interaction (see Figure 2C) indicates the TAF110 interaction is not sufficient for activation of transcription. Nevertheless, the ability of RXR to interact with two members of the TFIID complex that do not interact with each other (Hoey et al., *Cell* 72:247-260 (1993)) may be important for receptor function.

Accordingly, in accordance with the present invention, there are provided methods to identify compounds which are agonists or antagonists for retinoid X receptor (RXR). Invention method comprises:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain operatively associated with a transactivation dependent, ligand dependent component of the basal transcription machinery (or, alternatively, operatively associated with the RXR ligand binding domain),

a second fusion protein comprising the GAL4 activation domain, operatively associated with the RXR ligand binding domain (or, alternatively, operatively associated with a transactivation dependent, ligand dependent component of the basal transcription machinery),

said putative agonist or antagonist for RXR, and

a reporter construct comprising a GAL4 response element operatively linked to a reporter gene;

contacting:

a third fusion protein comprising the GAL4 DNA binding domain (or, alternatively, the GAL4

activation domain), operatively associated with a transactivation independent, ligand dependent component of the basal transcription machinery,

said second fusion protein (or, alternatively, said first fusion protein),

said putative agonist or antagonist for RXR, and

said reporter construct; and thereafter

identifying as agonists those compounds which induce transactivation in the presence of both said transactivation dependent, ligand dependent component and said transactivation independent, ligand dependent component of the basal transcription machinery,

identifying as antagonists those compounds which induce transactivation in the presence of said transactivation independent, ligand dependent component of the basal transcription machinery, but not in the presence of said transactivation dependent, ligand dependent component of the basal transcription machinery, and

identifying those compounds which fail to induce transactivation in the presence of either said transactivation dependent, ligand dependent component or said transactivation independent, ligand dependent component of the basal transcription machinery as neither agonists nor antagonists of hormone-mediated pathways involving RXR.

Optionally, compounds which fail to induce transactivation in the presence of either said transactivation dependent, ligand dependent component or said transactivation independent, ligand dependent component of the basal transcription machinery can be further tested for the ability to bind RXR. Those compounds which do not bind are neither agonists nor antagonists of RXR, while those compounds which bind RXR (but fail to induce transactivation thereof in the presence

of either of the above-described components of the basal transcription machinery) are presumably involved in other (i.e., non-hormone mediated) signalling pathways.

Various constructs employed in the practice of the present invention are well known in the art. Thus, the GAL4 DNA binding domain, the GAL4 activation domain, GAL4 resonse elements and various members of the basal transcription machinery have all been well characterized and extensively discussed in the art. For example, the DNA binding domain of the yeast GAL4 protein comprises at least the first 74 amino acids thereof (see, for example, Keegan et al., *Science* 231:699-704 (1986)). Preferably, the first 90 or more amino acids of the GAL4 protein will be used, with the first 147 amino acid residues of yeast GAL4 being presently most preferred.

The GAL4 fragment comprising the DNA binding domain employed in the practice of the present invention can be incorporated into any of a number of sites within the receptor protein. For example, the GAL4 DNA binding domain can be introduced at the amino terminus of the receptor protein, or the GAL4 DNA binding domain can be substituted for the native DNA binding domain of the receptor, or the GAL4 DNA binding domain can be introduced at the carboxy terminus of the receptor protein, or at other positions as can readily be determined by those of skill in the art.

Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:1),

such as, for example, 17MX, as described by Webster et al., in *Cell* 52:169-178 (1988), as well as derivatives thereof. Additional examples of suitable response elements include

those described by Hollenberg and Evans in *Cell* 55:899-906 (1988); or Webster et al. in *Cell* 54:199-207 (1988).

Numerous components of the basal transcription machinery have been described, e.g., TBP, TAF, TAF110, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, SUG1, TRIP1, TIF1, and the like.

Exemplary transactivation dependent/ligand dependent components of the basal transcription machinery include the TATA binding protein (TBP), SUG1, TRIP1, and the like.

An exemplary transactivation independent, ligand dependent component of the basal transcription machinery is the TBP mutant, TAF110.

Reporter constructs contemplated for use in the practice of the present invention comprise:

(a) a promoter that is operable in the host cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein the reporter protein-encoding DNA segment is operatively linked to the promoter for transcription of the DNA segment, and

wherein the hormone response element is operatively linked to the promoter for activation thereof.

Hormone response elements contemplated for use in the practice of the present invention are composed of at least one direct repeat of two or more half sites separated by a spacer of one nucleotide. The spacer nucleotide can be selected from any one of A, C, G or T. Each half site

of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Presently preferred response elements contain at least one copy (with one, two or three copies most common) of the minimal sequence:

AGGACA A AGGTCA (SEQ ID NO:2).

As noted above, the minimal sequence can optionally be flanked by additional residues, for example, as in the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ ID NO:3).

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in *Nature* 345:224-229 (1990), Mangelsdorf et al., in *Cell* 66:555-561 (1991), and Berger et al., in *J. Steroid Biochem. Molec. Biol.* 41:733-738 (1992)].

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented, for example, by the terms "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes, the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "GAL4 response element" was "turned on" or otherwise activated.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound.

"Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In the invention assay, the RXR-TBP interaction is ligand-dependent. Invention assays and transactivation experiments are carried out with ligand concentrations well above the K_d values for LG69 and 9-*cis* retinoic acid (Allegretto et al., *J. Biol. Chem.* 268:26625-26633 (1993)) such that small changes in ligand affinity would not be expected to have significant effects. The ability of RXR rc domain mutants to intaract in a ligand-dependent fashion with TAF110 (see Figure 2D) and to bind ligand *in vitro* indicates that the absence of an interaction between the mutants and TBP does not result from a defect in ligand binding.

Taken together, these results suggest the RXR rc domain directly interacts with TBP and that this interaction is regulated by ligand. This conclusion is supported by the *in vitro* interaction between the GAL4-rc domain fusion and TBP. Finally, the ability to recover the interaction between the RXR rc domain mutant and TBP by introducing a second site mutation in TBP (see Figure 3B) further supports the conclusion that the rc domain directly interacts with TBP. The ability of multiple factors to contact the basic repeat of TBP suggests that interaction with this domain of TBP may represent a common mechanism for transactivation.

The observation that RAR and TR do not interact with TBP (see Figure 2A) suggests that different RXR/nuclear receptor heterodimers may activate transcription by contacting different components of the transcriptional machinery. This conclusion is consistent with the observation that ligand responsiveness of RXR can be modified by heterodimeric pairing. The ability of

mutations in the RXR τc domain to adversely effect transactivation by heterodimers suggests that when complexed as a heterodimer, the RXR τc domain can be redirected to a different coactivator or component of the basal transcription machinery.

In accordance with another embodiment of the present invention, there are provided RXR mutants which retain the ability to bind 9-*cis*-retinoic acid, but which are not activated by 9-*cis*-retinoic acid. Examples of such mutants include RXR mutant D444A, RXR mutant T445A, RXR mutant P446A, RXR mutant I447A, RXR mutant D448A, RXR mutant T449A, RXR mutant F450P, RXR mutant L451A, RXR double mutant M454A, L455A, RXR double mutant E453K, E456K, RXR mutant M452A, and the like.

In accordance with yet another embodiment of the present invention, there are provided methods to identify agonists of retinoid X receptors. Invention method comprises:

contacting cells containing an RXR mutant as described above (i.e., having the ability to bind 9-*cis*-retinoic acid, but lacking the ability to be activated by 9-*cis*-retinoic acid) with a putative RXR ligand, wherein said cells contain an RXR response element operatively linked to a reporter gene, and thereafter

monitoring the expression of reporter gene product.

In an alternate aspect of this embodiment, there are also provided methods to identify antagonists of retinoid X receptors. This method comprises:

contacting cells containing an RXR mutant (as described above) with a constant amount of an RXR agonist and variable amounts of a putative antagonist therefor, wherein said cells contain an RXR response element operatively linked to a reporter gene, and thereafter

monitoring the expression of reporter gene product as a function of the amount of putative antagonist administered to said test cell.

In accordance with yet another embodiment of the present invention, there are provided methods to detect ligand-dependent interactions between retinoid X receptor and one or more components of the basal transcription machinery. Invention methods comprise:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain, operatively associated with a first component of the basal transcription machinery (or, alternatively, operatively associated with the RXR ligand binding domain),

a second fusion protein comprising the GAL4 activation domain, operatively associated with the RXR ligand binding domain (or, alternatively, operatively associated with a first component of the basal transcription machinery),

an RXR ligand, and

a reporter construct comprising a GAL4 response element operatively linked to a reporter gene; and thereafter

monitoring for expression of said reporter.

In accordance with a further aspect of this embodiment of the present invention, the above-described

contacting and monitoring steps can be repeated, employing a different first fusion protein (or different second fusion protein) which differs from the original first (second) fusion protein by containing a different component of the basal transcription machinery than the original first (second) fusion protein. This added step allows one to identify both transcription dependent/ligand dependent and transcription independent/ligand dependent components of the basal transcription machinery, which are useful for conducting the above-described assays.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1
Preparation of plasmids

For integration in *S. cerevisiae*, plasmid pRS305CYH was constructed by cloning a *BglII-SalI* fragment from pAS1-CYH2 (gift of S. Elledge, Baylor College of Medicine) containing the ADH promoter, GAL4 DNA binding domain (amino acids 1-147), influenza hemagglutinin epitope and poly linker in the order written into BamHI-SalI digested pRS305 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)).

For expression of GAL4-DNA binding domain fusions of human TBP mutants in *S. cerevisiae* (see below), plasmid pG6H was constructed by PCR amplification of the GAL4 DNA binding domain-influenza hemagglutinin epitope-polylinker from pAS1-CYH2. A sequence encoding six histidines was included directly after the initiator methionine of GAL4 in the 5' oligonucleotide. The amplified product was ligated into BamHI digested pG-1 (Schena et al., In: *Vectors for constitutive and inducible gene expression in yeast*, Guthrie and Fink (eds.), (Academic Press, Inc., San Diego) pp. 389-398 (1991)).

For expression of GAL4-DNA binding domain fusions in CV1 cells, the plasmid pCMXG4epi was constructed by PCR amplification of the GAL4 DNA binding domain-influenza hemagglutinin epitope-poly linker from pAS1-CHY2 and cloning into HindIII-BamHI digested pCMX (Umesono et al., *Cell* 65:1255-1266 (1991)). An optimal mammalian translation initiation sequence was included in the 5' oligonucleotide and introduced into the amino terminus of GAL4.

Receptor ligand binding domain fusions were cloned by PCR amplification of human RXRα (amino acids 197-462), human RARα (amino acids 186-462) and human TRα (amino acids 121-410). A ligand binding domain fusion with truncation of the τc domain (RXR197-443) was amplified as described above, however, an in-frame stop condon was introduced into the 3' oligonucleotide at the appropriate position.

For τc domain fusions, amino acids 444-462 of human RXRα and 391-410 of human TRα were amplified by PCR. Point mutations were introduced into the RXR τc domain by PCR using oligonucleotides with the appropriate base changes. Amplified products were ligated into NcoI-BamHI digested pRS305CYH. For expression in CV1 cells, appropriate restriction fragments from the pRS305CYH clones were subcloned into pCMXG4epi.

For two-hybrid assays, GAL4-activation domain fusions of RXR, RAR and TR were constructed by cloning the same amplification products described above into *NcoI-BamHI* digested pACTII (S. Elledge, Baylor College of Medicine; see Durfee et al., in *Genes & Development* 7:555-569 (1993)).

GAL4-DNA binding domain fusions expressing the C-terminal domain of human TBP (pAS+h180c), full length

Drosophila TAF110) and full length *Drosophila* TAF40 (pAS+dTAF40) were provided by G. Gill and R. Tjian (UC Berkeley; see Hoey et al., in *Cell* 72:247-260 (1993)). Human TBP (amino acids 155-335) was amplified by PCR in two fragments. Point mutations were introduced into the appropriate oligonucleotides. After PCR, the two fragments were cloned into *NcoI/BamHI* digested pG6H. A GAL4-DNA binding domain fusion of human TFIIB was made by PCR amplification of the human cDNA and cloned into the *BamHI* site of pG6H.

GST-RXR197-462 was constructed by PCR amplification of the appropriate sequences from human RXRα. The amplification products were cloned into *EcoRI-BamHI* digested pGEX2TK. All PCR-derived constructs were verified by sequencing. The plasmid pGEX-TBP was the kind gift of Dr. I. Verma (Salk Institute; see Kerr et al., in *Nature* 365:412-419 (1993)). Mammalian expression constructs expressing the ligand biding domains of human RXRα, human RARα and human TRβ have been described elsewhere (Forman et al., *Cell* 81:541-550 (1995)).

The luciferase reporter GAL3-TK-LUC containing three binding sites for GAL4 upstream of the TK promoter luciferase fusion was the gift of Dr. P.N. Rangarajan. GAL3-TK-LUC contains three copies of double-stranded GAL4 reponse element, cloned upstream of the TK promoter of TK-LUC at the *Hind*III site. TK-LUC is prepared as follows: the MTV-LTR promoter sequence was removed from the MTV-LUC plasmid described by Hollenberg and Evans in *Cell* 55:899-906 (1988) by *Hind*III and *Xho*I digest, and cloned with the *Hind*III-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in *Nucleic Acids Res.* 15:5490 (1987)) to generate parental construct TK-LUC.

Example 2
Yeast Strains and Methods

The strain Y190 (MATa gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3,-112 cyh^r URA3::GAL1-->lacZ LYS2::GAL1-->HIS3; a gift of S. Elledge, Baylor College of Medicine; Y190 is derived from Y153, described by Durfee et al., supra) was used for all experiments. For beta-galactosidase assays, a minimum of three independent transformants were grown overnight at 30°C in minimal media (0.66% YNB, 2% glucose) supplemented with the appropriate amino acids. Cells were diluted 1:20 into fresh media and 9-*cis* retinoic acid of 3,3',5-triiodthyroacetic acid (TRIAC) was added if required. Beta-galactosidase activity was measured after 16 hours of growth at 30°C as described by Rose et al., *Methods in yeast genetics* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor) (1990).

Example 3
Transfection

CV1 cells were plated in 48 well plates at a density of $2x10^4$ cells/well in DMEM supplemented with 10% charcoal-resin split fetal bovine serum. After growth at 37°C for 12-16 hours, cells were transfected using the DOTAP transfection reagent following the manufacturer's instructions (Boehringer Mannheim). For each well, 12ng of GAL3-TK-LUC reporter, 36ng of the appropriate expression constructs and as an internal control, 60ng of pCMX-βgal DNA were transfected. DNA was introduced along with 200 μl of DMEM supplemented with 10% charcoal-resin split fetal bovine serum. Cells were incubated with DNA for 5 hours at 37°C. The media was then removed, the cells washed once with fresh media and 200 μl of media with or without 9-*cis* retinoic acid, T_3 (3,3',5-triiodo-L-thyronine) or vitamin D_3 was added. The RXR specific ligand LG69 (4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl-1-ethenyl]benzoic

acid) and the RAR specific ligand AM580 (4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthamido)benzoic acid) were also used. Cells were harvested after 36 hours of growth at 37°C. Luciferase activity of each sample was normalized by the level of beta-galactosidase activity. Each transfection was carried out in duplicate and repeated at least three times. The fold induction reported is relative to the GAL3-TK-LUC reporter alone included in each experiment.

Example 4
RXR-TBP interaction Assay

GST-fusion proteins were induced, solubilized and bound to glutathione beads following the manufacturer's procedures (LKB-Pharmacia). After binding to glutathione beads, 15 µl of the suspension was incubated with 1 to 2 µl of the appropriate ^{35}S-labeled *in vitro* translated protein for 1 hour in 500 µl of NETN (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 0.7 mM EDTA, 0.5% NP40, 1 mM PMSF). Following incubation, the beads were washed three times with NETN. Bound proteins were eluted with 20 µl of 1X SDS-PAGE buffer and electrophoretically separated in a SDS-10% polyacrylamide gel. The interaction of *in vitro* translated GAL4 fusions with GST-TBP was carried out using the above procedure with the following modifications. The initial interaction was carried out in NETN in which KCl was replaced with 0.3M NaCl and non-fat dry milk was added to final concentration of 0.5% (w/v). Following incubation, the beads were washed three times with NETN in which the NaCl concentration was increased to 0.5M and non-fat dry milk was added to final concentration of 0.5% (w/v). Following fixation gels were treated with 1 M salicylic acid, dried and subjected to autoradiography.

Example 5
Mutagenesis of the RXR τc domain

The carboxy terminal 19 amino acids of RXR and 20 amino acids of TR have been shown to activate transcription when fused to heterologous DNA binding domains (Figure 1; (Durand et al., supra; Leng et al., supra; Zhang et al., supra; Baniahmad et al., *Mol. Cell. Biol.* 15:76-86 (1995); Barettino et al., *EMBO J.* 13:3039-3049 (1994); and Tone et al., *J. Biol. Chem.* 269:31157-31161 (1994)). This region has been proposed to form an amphipathic alpha helix with hydrophobic and negatively charged faces (Danielian et al., supra).

Figure 1A presents transactivation results with fusions between the GAL4 DNA binding domain and mutants of the last 19 amino acids of human RXRα (amino acids 444-462) and the last 20 amino acids of human TRα (amino acids 391-410). These constructs were transfected into CV1 cells along with the reporter GAL3-TK-LUC or integrated into the genome of the *S. cerevisiae* strain Y190 containing the integrated GAL1-lacZ reporter as described above. CV1 cell transfection results were normalized by cotransfection with a beta-galactosidase expression plasmid. Western blotting of *S. cerevisiae* extracts indicates the GAL4 fusions are expressed at similar levels.

Mutation of the carboxy-terminal 19 amino acids of RXR (Figure 1A) indicates that, like several other transactivation domains, the hydrophobic and acidic amino acids are critically important for function (Cress and Triezenberg, *Science* 251:87-90 (1991)). Within the hydrophobic face of the helix individual changes of phenylalanine at position 450 to proline (F450P), leucine at position 451 to alanine (L451A) and the double mutant methionine 454 to alanine/leucine 455 to alanine (M454A/L455A) severely reduce the ability of GAL4 fusions

to activate transcription when assayed in the context of the isolated rc domain in both mammalian and *S. cerevisiae* cells. The double mutant glutamic acid 453 to lysine/glutamic acid 456 to lysine (E453K/E456K) on the charged face of the helix also eliminates the ability to the isolated rc domain to activate transcription (Figure 1A). The single mutations E453K and E456K reduce transcription approximately 60-70%.

Mutation of methionine 452 to alanine (M452A), however, has little effect. Incorporation of these same mutations into the complete ligand binding domain (Figure 1B) or into full length receptors reduces the ability of these mutant RXRs to activate transcription in response to RXR specific ligands. Importantly, truncation of the 19 amino acids (GAL4RXR197-443) also produces a receptor that fails to activate transcription (Figure 1B). The reduction in ligand-dependent transcription observed with GAL4RXR197-443 does not appear to result from a defect in ligand binding (see Figure 2D). Taken together, the results confirm that the last 19 amino acids of RXR are both necessary and sufficient for transactivation and indicate that both the hydrophobic and charged faces of the helix residues are important for this function.

Example 6

RXR interacts with the TATA binding protein

The finding that mutations in the RXR rc domain have qualitatively similar effects in mammalian and *S. cerevisiae* cells (Figure 1A) suggests that RXR directly contacts a structurally and functionally conserved component of the transcription machinery. This observation is consistent with the finding that several other transcription factors, including members of the steroid and thyroid hormone receptor superfamily, interact with components of the basal transcription machinery (Ing et

al., supra; Baniahmad et al., (1993) supra; Fondell et al., supra; Blanco et al., supra; and MacDonald et al., supra). Therefore, we examined the interactions between RXR and several basal transcription factors, including the TATA binding protein (TBP), TAF110, TAF40 and TFIIB, using the both yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989); and Durfee et al., *Genes & Devel.* 7:555-569 (1993)) and *in vitro* protein-protein interaction assays. As shown in Figure 2A, the two-hybrid assay detects a specific and ligand-dependent interaction between RXR and the conserved carboxy-terminal domain of TBP.

Thus, fusions between the GAL4 activation domain and RXR, RAR, and TR and RXR rc mutants were cotransformed into the strain Y190 along with fusions between the GAL4 DNA binding domain and the conserved carboxy terminal domain of human TBP (see Figures 2A and 2B) or full length *Drosophila* TAF110 (see Figures 2C and 2D). Beta-galactosidase activity was measured after growth for 16 hours in the presence (filled bars) or absence (open bars) of 1 μM 9-*cis* retinoic acid (RXR and RAR) or 1 μM TRIAC (TR; see Figures 2A and 2C). Interaction between receptor ligand binding domains and TBP and TAF110 are shown in Figure 2A and 2C, respectively. The activity of the GAL4 activation domain alone was measured only in the absence of ligand. Note the difference in scale between Figures 2A and 2C.

The interaction between RXR ligand binding domain mutants and TBP and TAF110 are shown in Figures 2B and 2D, respectively. Only activity in the presence of 9-*cis* retinoic acid is shown. No interactions between the mutants and TBP or TAF110 is detected in the absence of 9-*cis* retinoic acid. Point mutants consist of amino acids 197-462 of RXR. RXR197-443 represents the rc truncation. Western blotting of *S. cerevisiae* extracts indicates the

GAL4-activation domain fusions are expressed at similar levels. Interactions between TAF40 and RXR, RAR or TR were also tested and not detected. An interaction was detected between TR and TFIIB.

Mutations in the RXR τc domain that eliminate the ability of RXR to activate transcription (Figure 1) eliminate a detectable interaction between RXR and TBP (Figure 2B). Although TR and RAR have τc domains that exhibit significant sequence homology to the RXR τc domain, an interaction between TR or RAR and TBP is not detected (Figure 2A). Nevertheless, the same region of TR activates transcription in *S. cerevisiae* when fused to the GAL4 DNA binding domain. The failure to detect an interaction between RAR and TBP or between TR and TBP suggests that transactivation by RXR homodimers may utilize different components of the transcription machinery than transactivation by RAR and TR heterodimers.

Figure 2C also shows that RXR can make a ligand-dependent interaction with a second component of the TFIID complex, TAF110. The interaction between RXR and TAF110 is detectable even when τc domain mutants are analyzed, indicating the functional state of the τc domain is not important for the interaction (Figure 2D). Nevertheless, the ability to detect ligand-dependent interactions between transcriptionally defective RXR mutants and TAF110 suggests that mutations in the RXR τc domain do not have large effects on ligand binding. The observation that TR (Figure 2C) also interacts with TAF110 suggests this basal factor may be a common target for multiple nuclear receptors.

Although the results of the two-hybrid assay suggest RXR makes a direct protein-protein interaction with TBP, the possibility that this interaction is mediated by a conserved coactivator cannot be ruled out by this assay. To further characterize the interaction between RXR and

TBP, the ability of TBP to interact *in vitro* with bacterial expressed glutathione-S-transferase RXR fusion proteins was examined.

Thus GST pull-down experiments were carried out as follows. TBP was *in vitro* transcribed and translated as described above, and incubated with equal amounts of immobilized GST-RXR197-462 or GST-RXR-E453K/E456K) as determined by coomassie stained gels. Following extensive washing of the beads, bound proteins were eluted and resolved by SDS-PAGE and the gel was processed for autoradiography. When added, 1.0 μM 9-*cis* retinoic acid was included in all buffers. Exposure time was 2 hours. Little or no interaction between TBP and GST alone is detected under these conditions.

Thus, the GST pull-down experiment shows a strong interaction between *in vitro* translated TBP and GST-RXR197-462. An *in vitro* interaction between GST-RXR197-462 and TAF110 is also observed. A mutation of the RXR rc domain (E453K/E456K) that eliminates the RXR-TBP interaction in the two-hybrid assay (Figure 2B) reduces the *in vitro* interaction between RXR and TBP approximately 6 fold. Similar results are observed when a full length GST-RXR fusion is used.

A direct *in vitro* interaction between TBP and the rc domain itself (GAL4RXR444-462) that is sensitive to the functional state of the rc domain can also be detected, as follows. Equal amounts of *in vitro* translated GAL4RXR444-462, GAL4RXR444-462-E453K/E456K or GAL4(1-147) as determined by phosphorimaging analysis were incubated with immobilized GST-TBP or with immobilized GST. Following extensive washing of the beads, bound proteins were eluted and resolved by SDS-PAGE and the gel was processed for autoradiography. Exposure time was 7 hours. The sensitivity of the *in vitro* interactions to mutations

in the RXR τc domain strongly suggests the τc domain mediates a direct interaction between RXR and TBP.

Unlike the two hybrid assay, a RXR-TBP interaction *in vitro* can be detected in the absence of ligand. Addition of ligand stimulates the interaction 3-5 fold when quantitated by phosphorimaging. The detection of ligand-independent interactions *in vitro* may result from the ability of the large amounts of protein used *in vitro* to stabilize a weak interaction that cannot be detected in the two-hybrid assay.

To further define the RXR-TBP interaction, mutations were introduced into well conserved amino acids present in the basic repeat of TBP and analyzed for interaction with RXR in the two-hybrid assay. This domain of TBP has been shown to be a common target of several transcription factors (Lee et al., *Cell* 67:365-376 (1991); Metz et al., *Mol. Cell. Biol.* 14:6021-6029 (1994a); and Metz et al., *EMBO J.* 13:3832-3842 (1994b)). Thus, a Fusion between the GAL4 activation domain and RXR (amino acids 197-462) was cotransformed into the strain Y190 along with fusions between the GAL4 DNA binding domain and human TBP (the conserved carboxy terminal domain, amino acids 151-335). Y233G, R321E/K232E/R235E, V236G and V237G identify the amino acid changes introduced into TBP. Beta-galactosidase activity was measured after growth for 16 hours in the presence of 1 μM 9-*cis* retinoic acid as described in Materials and Methods. Western blotting of *S. cerevisiae* extracts indicates the GAL4-TBP fusions were expressed in similar levels.

Figure 3A shows that the TBP mutant V237G eliminates a detectable RXR-TBP interaction. Several other mutations in this region of TBP, including V236G, have no effect.

The finding that a single point mutation in TBP could disrupt the interaction with the wildtype RXR ligand binding domain prompted an examination of the ability of TBP-V237G to interact with the RXR rc mutants. Thus, fusions between the GAL4 activation domain and RXR rc mutants were cotransformed into the strain Y190 along with a fusion between the GAL4 DNA binding domain and the TBP mutants described in reference to Figure 3A. Beta-galactosidase activity was measured after growth for 16 hours in the presence (filled bars) or absence (open bars) of 1 uM 9-*cis* retinoic acid as described above. Point mutants consist of amino acids 197-462 of RXR. RXR197-443 represents the rc truncation. Note the difference in scale between A and B.

As shown in Figure 3B, a positive and ligand-dependent interaction can be detected between TBP-V237G and a single RXR rc domain mutant, M454A/L455A. Although the interaction detected between TBP-V237G and RXR-M454A/L455A is weak relative to the wildtype interaction, an approximate 10-fold ligand-dependent induction of the interaction is observed (Figure 3B). Rescue of the RXR-TBP interaction by combining a RXR rc domain mutant with a TBP mutant strongly suggests the RXR-TBP interaction detected in the two-hybrid assay results from a direct protein-protein interaction and is not mediated by a third factor.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

SEQ ID NO:1:

5'-CGGAGGACTGTCCTCCG-3'

SEQ ID NO:2:

5'-AGGACA A AGGTCA-3'

SEQ ID NO:3:

5'-GGACC AGGACA A AGGTCA CGTTC-3'

That which is claimed is:

1. A method to identify compounds which are agonists or antagonists for retinoid X receptor (RXR), said method comprising:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain, operatively associated with a transactivation dependent, ligand dependent component of the basal transcription machinery,

a second fusion protein comprising the GAL4 activation domain, operatively associated with the RXR ligand binding domain,

said putative agonist or antagonist for RXR, and

a reporter construct comprising a GAL4 response element operatively linked to a reporter gene;

contacting:

a third fusion protein comprising the GAL4 DNA binding domain, operatively associated with a transactivation independent, ligand dependent component of the basal transcription machinery,

said second fusion protein,

said putative agonist or antagonist for RXR, and

said reporter construct; and thereafter

identifying as agonists those compounds which induce transactivation in the presence of both said transactivation dependent, ligand dependent component and said transactivation independent, ligand dependent component of the basal transcription machinery,

identifying as antagonists those compounds which induce transactivation in the presence of said

transactivation independent, ligand dependent component of the basal transcription machinery, but not in the presence of said transactivation dependent, ligand dependent component of the basal transcription machinery, and

identifying those compounds which fail to induce transactivation in the presence of either said transactivation dependent, ligand dependent component or said transactivation independent, ligand dependent component of the basal transcription machinery as neither agonists nor antagonists of RXR.

2. A method according to claim 1 wherein said transactivation dependent, ligand dependent component of the basal transcription machinery is TBP.

3. A method according to claim 2 wherein said transactivation independent, ligand dependent component of the basal transcription machinery is TAF110.

4. A method to identify compounds which are agonists or antagonists for retinoid X receptor (RXR), said method comprising:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain, operatively associated with the RXR ligand binding domain,

a second fusion protein comprising the GAL4 activation domain, operatively associated with a transactivation dependent, ligand dependent component of the basal transcription machinery,

said putative agonist or antagonist for RXR, and

a reporter construct comprising a GAL4 response element operatively linked to a reporter gene;

contacting:

said first fusion protein,

a third fusion protein comprising the GAL4 activation domain, operatively associated with a transactivation independent, ligand dependent component of the basal transcription machinery,

said putative agonist or antagonist for RXR, and

said reporter construct; and thereafter

identifying as agonists those compounds which induce transactivation in the presence of both said transactivation dependent, ligand dependent component and said transactivation independent, ligand dependent component of the basal transcription machinery,

identifying as antagonists those compounds which induce transactivation in the presence of said transactivation independant, ligand dependent component of the basal transcription machinery, but not in the presence of said transactivation dependent, ligand dependent component of the basal transcription machinery, and

identifying those compounds which fail to induce transactivation in the presence of either said transactivation dependent, ligand dependent component or said transactivation independent, ligand dependent component of the basal transcription machinery as neither agonists nor antagonists of RXR.

5. A method according to claim 4 wherein said transactivation dependent, ligand dependent component of the basal transcription machinery is TBP.

6. A method according to claim 5 wherein said transactivation independent, ligand dependent component of the basal transcription machinery is TAF110.

7. An RXR mutant which retains the ability to bind 9-*cis*-retinoic acid, but which is not activated by 9-*cis*-retinoic acid.

8. A mutant receptor according to claim 7, wherein said mutant is selected from RXR mutant D444A, RXR mutant T445A, RXR mutant P446A, RXR mutant I447A, RXR mutant D448A, RXR mutant T449A, RXR mutant F450P, RXR mutant L451A, RXR double mutant M454A, L455A, RXR double mutant E453K, E456K or RXR mutant M452A.

9. A method to identify agonists of retinoid X receptors, said method comprising:

contacting cells containing an RXR mutant according to claim 7 with a putative RXR ligand, wherein said cells contain an RXR response element operatively linked to a reporter gene, and thereafter

monitoring the expression of reporter gene product.

10. A method to identify antagonists of retinoid X receptors, said method comprising:

contacting cells containing an RXR mutant with a constant amount of an RXR agonist and variable amounts of a putative antagonist therefor, wherein said mutant retains the ability to bind 9-*cis*-retinoic acid, but is not activated by 9-*cis*-retinoic acid, wherein said cells contain an RXR response element operatively linked to a reporter gene, and thereafter

monitoring the expression of reporter gene product as a function of the amount of putative antagonist administered to said test cell.

11. A fusion protein comprising the GAL4 activation domain, operatively associated with the RXR ligand binding domain.

12. A method to detect ligand-dependant interactions between retinoid X receptor and one or more components of the basal transcription machinery, said method comprising:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain, operatively associated with a first component of the basal transcription machinery,

a second fusion protein comprising the GAL4 activation domain, operatively associated with the RXR ligand binding domain,

an RXR ligand, and

a reporter construct comprising a GAL4 response element operatively linked to a reporter gene; and thereafter

monitoring for expression of said reporter.

13. A method according to claim 12 further comprising repeating said contacting and monitoring steps, employing a different first fusion protein which differs from the original first fusion protein by containing a different component of the basal transcription machinery than the original first fusion protein.

14. A method according to claim 13 wherein said component of the basal transcription machinery is selected from TBP, TAF, TAF110, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, SUG1, TRIP1 or TIF1.

15. A method to detect ligand-dependent interactions between retinoid X receptor and one or more components of the basal transcription machinery, said method comprising:

contacting:

a first fusion protein comprising the GAL4 DNA binding domain, operatively associated with the RXR ligand binding domain,

a second fusion protein comprising the GAL4 activation domain, operatively associated with a first component of the basal transcription machinery,

an RXR ligand, and

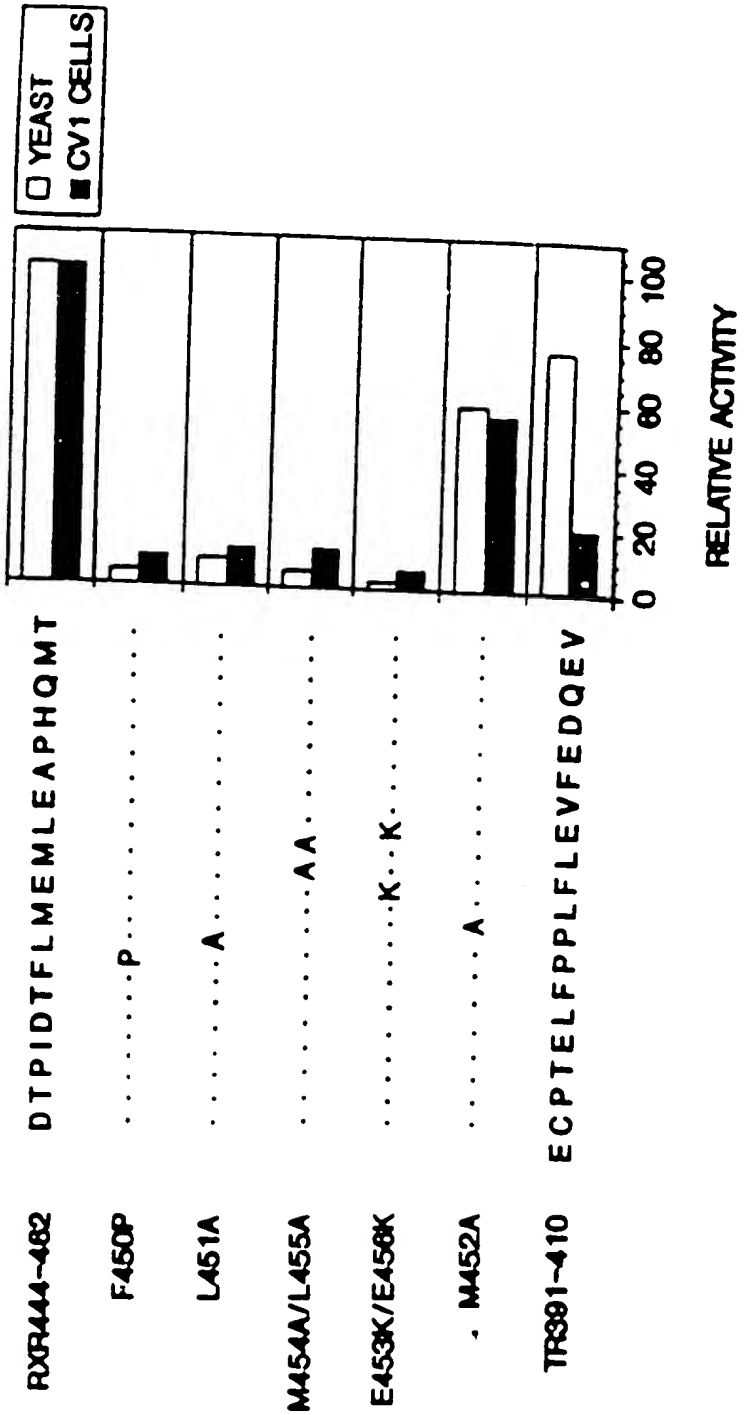
a reporter construct comprising a GAL4 response element operatively linked to a reporter gene; and thereafter

monitoring for expression of said reporter.

16. A method according to claim 15 further comprising repeating said contacting and monitoring steps, employing a different second fusion protein which differs from the original second fusion protein by containing a different component of the basal transcription machinery than the original second fusion protein.

17. A method according to claim 16 wherein said component of the basal transcription machinery is selected from TBP, TAF, TAF110, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, SUG1, TRIP1 or TIF1.

FIG. 1A


YEAST
CV1 CELLS
RELATIVE ACTIVITY
0 20 40 60 80 100
RXR444-462 DTPIDTFLMEMLEAPHQMT
F450P .....P.....
L451A .....A.....
M454A/L455A .....AA.....
E458K/E458K .....K..K.....
M452A .....A.....
TR391-410 ECPTELFPPLFLEVFEDQEV

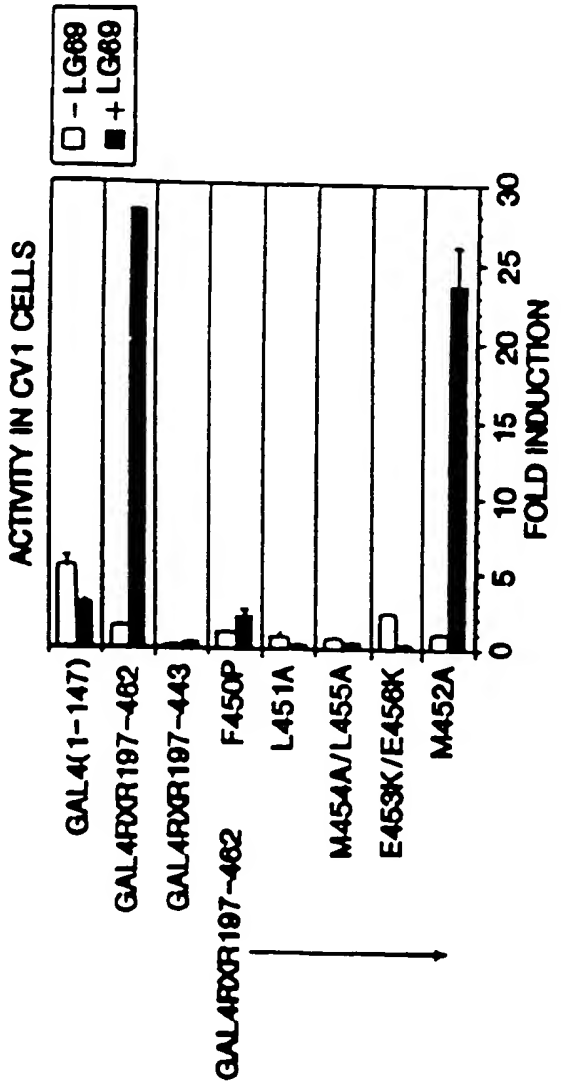

- LG69
+ LG69
ACTIVITY IN CV1 CELLS
FOLD INDUCTION
0
5
10
15
20
25
30
GAL4(1-147)
GAL4RXR197-462
GAL4RXR197-443
GAL4RXR197-462
F450P
L451A
M454A/L455A
E453K/E456K
M452A

FIG. 1B

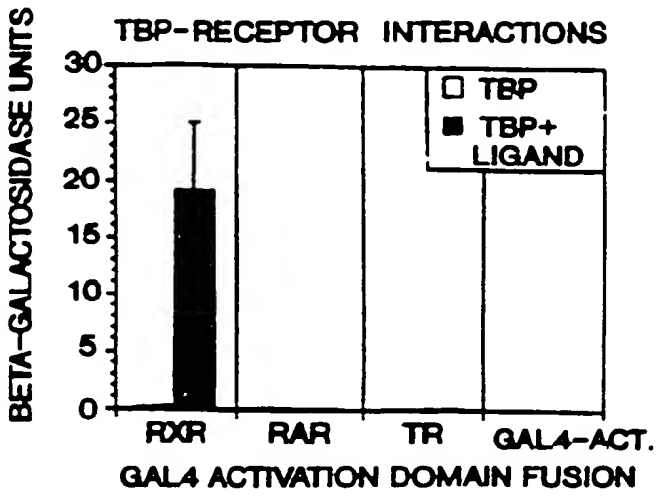

TBP-RECEPTOR INTERACTIONS
BETA-GALACTOSIDASE UNITS
30
25
20
15
10
5
0
TBP
TBP+ LIGAND
RXR
RAR
TR
GAL4-ACT.
GAL4 ACTIVATION DOMAIN FUSION

FIG. 2A

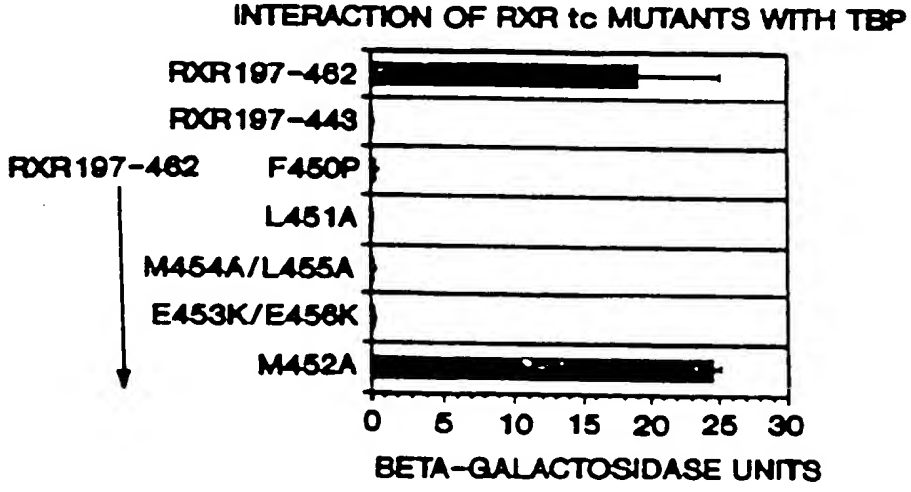

INTERACTION OF RXR tc MUTANTS WITH TBP
RXR197-462
RXR197-462
RXR197-443
F450P
L451A
M454A/L455A
E453K/E456K
M452A
0
5
10
15
20
25
30
BETA-GALACTOSIDASE UNITS

FIG. 2B

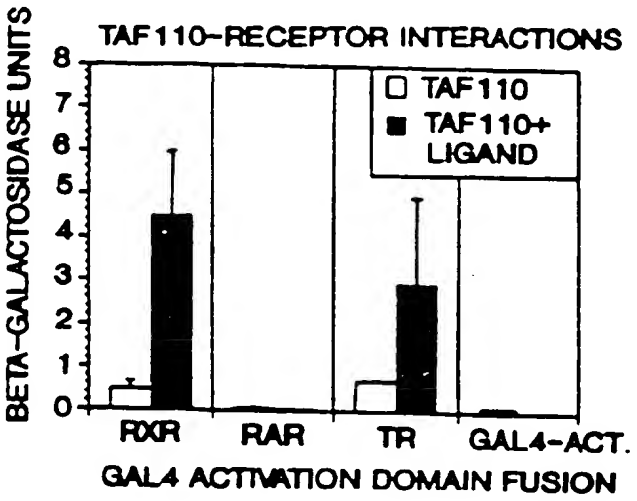

TAF110-RECEPTOR INTERACTIONS
BETA-GALACTOSIDASE UNITS
0
1
2
3
4
5
6
7
8
TAF110
TAF110+ LIGAND
RXR
RAR
TR
GAL4-ACT.
GAL4 ACTIVATION DOMAIN FUSION

FIG. 2C

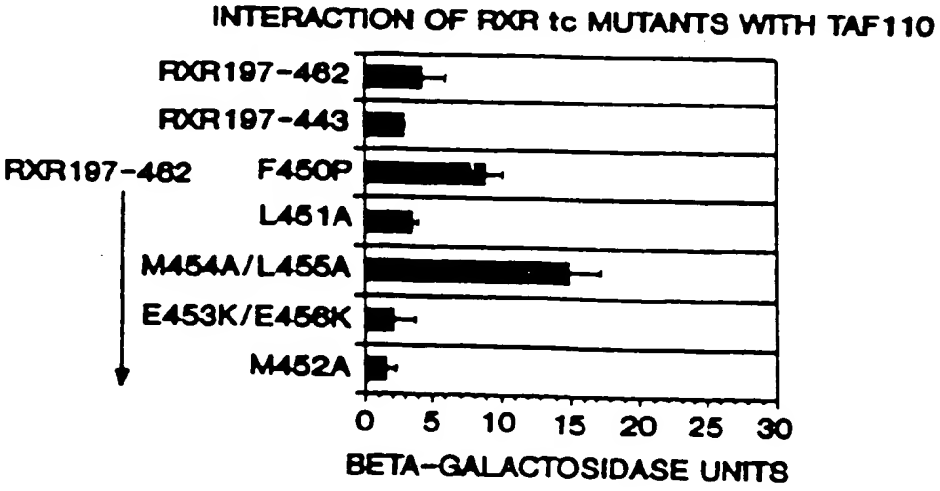

INTERACTION OF RXR tc MUTANTS WITH TAF110
RXR197-462
RXR197-443
RXR197-462 F450P
L451A
M454A/L455A
E453K/E456K
M452A
0
5
10
15
20
25
30
BETA-GALACTOSIDASE UNITS

FIG. 2D

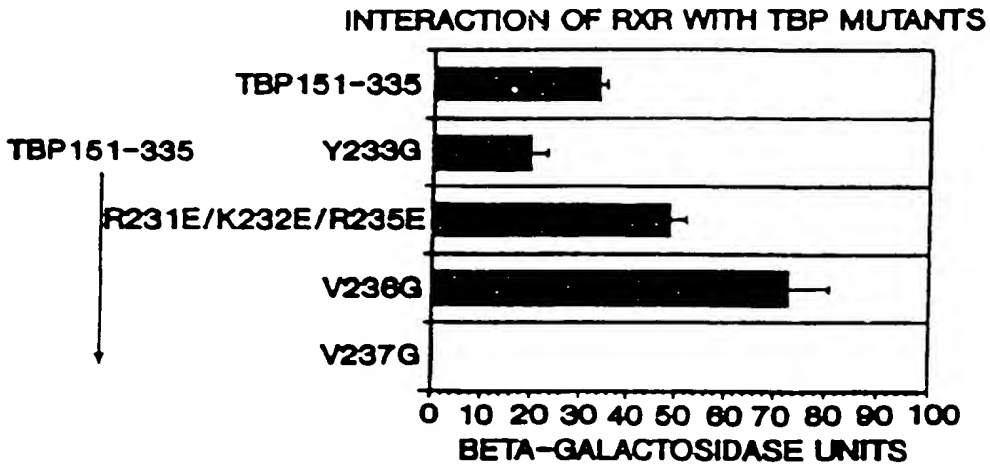

INTERACTION OF RXR WITH TBP MUTANTS
TBP151-335
TBP151-335
Y233G
R231E/K232E/R235E
V236G
V237G
0 10 20 30 40 50 60 70 80 90 100
BETA-GALACTOSIDASE UNITS

FIG. 3A

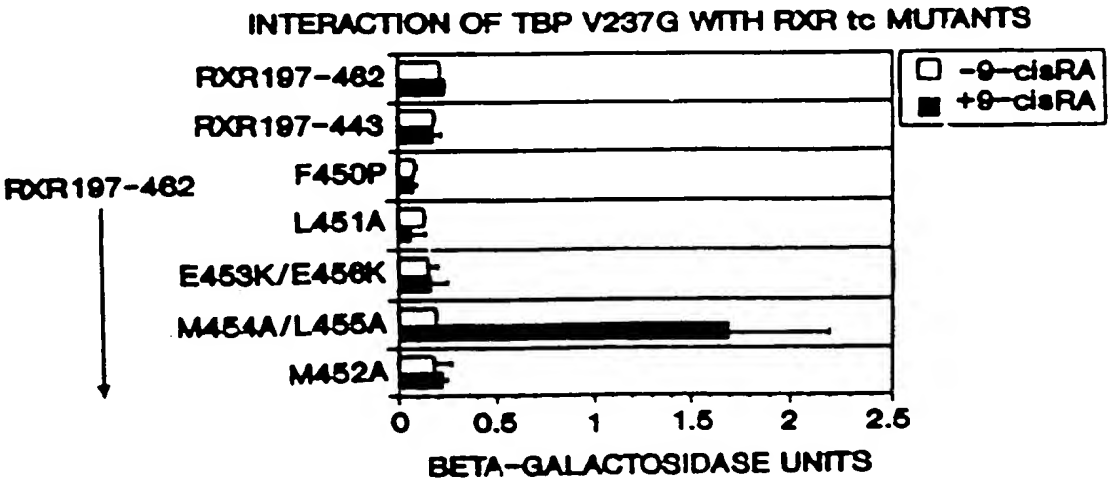

INTERACTION OF TBP V237G WITH RXR tc MUTANTS
-9-cisRA
+9-cisRA
RXR197-462
RXR197-443
RXR197-462
F450P
L451A
E453K/E456K
M454A/L455A
M452A
0 0.5 1 1.5 2 2.5
BETA-GALACTOSIDASE UNITS

FIG. 3B